AMENDMENTS TO THE CLAIMS:

Please amend claims 1, 28 and 30, and add claims 90 and 91 as follows. This listing of claims replaces all prior versions, and listings of claims in the application.

LISTING OF CLAIMS:

1. (Currently Amended) A high throughput method for generating a protein or peptide molecule having a predetermined property or activity, ~~the method~~ comprising:

(a) identifying, within a target protein or peptide, ~~one or more~~ a plurality of target amino acids amenable to providing the predetermined property or activity upon amino acid replacement, wherein:

the identifying of the one or more target amino acids in step a) is conducted *in silico*; and

each target amino acid locus is designated an *in silico*-HIT (is-HIT);

(b) identifying replacement amino acids to replace the residue at each is-HIT, wherein:

~~the replacement amino acids are amenable to providing the evolved predetermined property or activity to the target protein upon amino acid replacement; and~~

the replacement amino acids comprise all of the 19 remaining non-native amino acids or a restricted subset of amino acids up to all 19 remaining amino acid; and

a restricted subset is a group of ~~selected~~ amino acids selected to have a predetermined effect on protein activity;

(c) producing a collection of sets of nucleic acid molecules that encode candidate LEAD proteins, wherein:

each encoded candidate LEAD protein contains a single amino acid replacement;

each nucleic acid molecule in a set encodes the same candidate LEAD protein;

each candidate LEAD protein differs by one amino acid from the target protein or peptide, whereby the encoded candidate LEAD proteins in each set differ by one amino acid from the encoded candidate LEAD proteins in each of the other sets;

each set is separate from each and all other sets;

(d) individually introducing each set of nucleic acid molecules into host cells and expressing the encoded candidate LEAD proteins to produce sets of LEAD proteins, whereby:

each candidate LEAD protein in a set contains the same amino acid replacement;

the host cells comprise an addressable array such that each LEAD protein is expressed at a different locus in the array, and the identity of each candidate LEAD protein at each locus is known; and

(e) individually screening each set of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity of an unmodified target protein, wherein each such identified protein is designated a LEAD mutant protein.

2. (Original) The method of claim 1, wherein the array comprises a solid support with separate loci and each set of cells is at a different locus.

3. (Original) The method of claim 2, wherein the loci comprise wells; and each well contains one set of cells.

4. (Original) : The method of claim 1, wherein the nucleic acid molecules comprise plasmids; and the cells are eukaryotic cells that are transfected with the plasmids or are bacterial cells are transformed with the plasmids.

5. (Original) The method of claim 1, wherein the nucleic acid molecules in step (c) are produced by site-specific mutagenesis.

6. (Previously Presented) The method of claim 1, further comprising:

(f) generating a population of sets nucleic acid molecules encoding a population of sets of candidate super-LEAD proteins, wherein:

a candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins;

each set of nucleic acid molecules encodes a single candidate LEAD protein;

(g) separately introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells, and expressing the encoded candidate super-LEAD proteins to produce sets of candidate super-LEAD proteins; and

(h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

7. (Original) The method of claim 6, wherein the nucleic acid molecules in step (f) are produced by a method selected from among Additive Directional Mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and *de novo* synthesis.

8. Cancelled.

9. (Original) The method of claim 1, wherein the replacement amino acids identified in step (b) correspond to a restricted subset of the 19 remaining non-native amino acids.

10. (Original) The method of claim 1, wherein the nucleic acids of step (c) are produced by systematically replacing each codon that is an is-HIT, with one or more codons encoding a restricted subset of the remaining amino acids, to produce nucleic acid molecules each differing by at least one codon and encoding candidate LEADs.

11. (Original) The method of claim 6, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

12. (Withdrawn) The method of claim 1, wherein the change in activity is at least about 10% of the activity of the unmodified target protein.

13. (Withdrawn) The method of claim 1, wherein the change in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

14. (Withdrawn) The method of claim 1, wherein the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

15. (Original) The method of claim 1, wherein the activity modified is selected from among increased catalytic activity, altered substrate and ligand recognition, increased thermostability, increased stability, increased resistance to proteases, increased resistance to glomerular filtration, increased immunogenicity, increased cationization, increased anionization and pseudo wild-type function.

16. (Original) The method of claim 1, wherein each is-HIT target amino acid is susceptible to digestion by one or more proteases.

17. (Previously Presented) The method of claim 16, wherein the LEADs possess increased resistance to proteolysis compared to unmodified target protein.

18. (Previously Presented) The method of claim 1, wherein each is-HIT target amino acid is resistant to digestion by one or more proteases compared to in unmodified protein.

19. (Withdrawn) The method of claim 18, wherein the LEADs possess increased digestibility compared to unmodified target protein.

20. (Withdrawn) The method of claim 1, wherein each is-HIT target amino acid affects protein conformation and/or antigenicity.

21. (Withdrawn) The method of claim 20, wherein the LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

22. (Withdrawn) The method of claim 1, wherein each is-HIT target amino acid affects protein amphipathic properties.

23. (Withdrawn) The method of claim 22, wherein the LEADs or super-LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

24. (Withdrawn) The method of claim 1, wherein each is-HIT target amino acid is amenable to constitute a link or bridge between two regions of a protein.

25. (Withdrawn) The method of claim 24, wherein the LEADs possess increased thermostability compared to unmodified target protein.

26. (Withdrawn) The method of claim 1, wherein each is-HIT target amino acid affects binding affinity to its cognate receptor.

27. (Withdrawn) The method of claim 26, wherein the LEADs possess either increased or decreased binding affinity to the LEADs or super-LEADs cognate receptor compared to unmodified target protein.

28. (Currently Amended) A high throughput method for generating proteins with a desired property or activity, comprising:

(a) identifying residues in a target protein *in silico* that are associated with the property, and designating the loci of such residues as is-HIT target loci;

(b) preparing variant nucleic acid molecules encoding variant proteins, wherein:

each variant nucleic acid encodes a candidate LEAD mutant protein that differs by one replacement amino acid at one is-HIT target locus from the target protein; ~~, wherein:~~

the amino acid residues at each of the identified is-HIT target loci in the target protein is replaced with all of the non-native amino acids, or the amino acid residues at each of the identified is-HIT target loci in the target is replaced with a restricted subset of the remaining 19 non-native amino acids; and

a restricted subset is a group of ~~selected~~ amino acids selected to have a predetermined effect on protein activity;

(c) separately introducing the nucleic acid molecules encoding each candidate LEAD protein into hosts for expression thereof, and expressing the nucleic acid molecules encoding each variant protein to produce sets of candidate LEAD proteins, wherein:

each candidate LEAD protein in a set contains the same amino acid replacement;

each candidate LEAD protein contains a single amino acid replacement, and differs from the target protein by one amino acid replacement; and

each replacement is at the same locus; and

(d) individually screening each set of variant LEAD candidate proteins to identify any that have an activity or property that differs by a predetermined amount from the activity of the unmodified target protein, thereby identifying proteins that are LEADs.

29. Cancelled.

30. (Currently Amended) The method of claim 28, wherein each of the residues at identified is-HIT loci in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids~~, wherein a restricted subset is a group of selected amino acids selected to have a predetermined effect on protein activity~~.

31. (Previously Presented) The method of claim 28, wherein the total number of is-HIT loci that are replaced with replacement amino acids is less than the total number of amino acid residues within the full-length of the target protein.

32. (Previously Presented) The method of claim 28, wherein each of the residues at identified is-HIT loci in the target protein is replaced with a restricted subset of the remaining 19 amino acids; and the total number of is-HIT loci that is replaced with replacement amino acids is less than the total number of amino acid residues within the full-length of the target protein.

33. (Previously Presented) The method of claim 28, further comprising:

(e) generating a population of sets of nucleic acid molecules encoding sets of candidate super-LEAD proteins, wherein:

each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins; and

each set encodes a single candidate super-LEAD protein;

(f) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and

(g) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

34. (Previously Presented) The method of claim 33, wherein the nucleic acid molecules in step (e) are produced by a method selected from among additive directional mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and *de novo* synthesis.

35. (Original) The method of claim 33, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

36. (Original) The method of claim 28, wherein each is-HIT target residue is susceptible to digestion by one or more proteases.

37. (Previously Presented) The method of claim 36, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

38. (Original) The method of claim 28, wherein each is-HIT target residue is resistant to digestion by one or more proteases.

39. (Withdrawn) The method of claim 38, wherein the LEADs or super-LEADs possess increased digestibility compared to unmodified target protein.

40. (Withdrawn) The method of claim 28, wherein each is-HIT target residue affects protein conformation.

41. (Withdrawn) The method of claim 40, wherein the LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

42. (Withdrawn) The method of claim 28, wherein each is-HIT target amino acid affects protein amphipathic properties.

43. (Withdrawn) The method of claim 42, wherein the LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

44. (Withdrawn) The method of claim 28, wherein each is-HIT target amino acid is amenable to constitute a link or bridge between two regions of a protein.

45. (Withdrawn) The method of claim 44, wherein the LEADs possess increased thermostability compared to unmodified target protein.

46. (Withdrawn) The method of claim 28, wherein each is-HIT target amino acid affects binding affinity to its cognate receptor.

47. (Withdrawn) The method of claim 46, wherein the LEADs possess either increased or decreased binding affinity to the LEADs or super-LEADs cognate receptor compared to unmodified target protein.

48. (Withdrawn) The method of claim 28, wherein the change in activity is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

49. (Withdrawn) The method of claim 28, wherein the change in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

50. (Withdrawn) The method of claim 28, wherein the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

51. Cancelled.

52. Cancelled.

53. (Previously Presented) The method of claim 1, wherein:

the predetermined property is susceptibility to digestion by one or more proteases.

54. (Original) The method of claim 53, wherein the LEADs possess increased resistance to proteolysis compared to unmodified target protein.

55. (Previously Presented) The method of claim 1, wherein:

the predetermined property is resistance to digestion by one or more proteases.

56. (Withdrawn) The method of claim 55, wherein the LEADs possess increased digestibility compared to unmodified target protein.

57. (Withdrawn, Currently Amended) The method of claim 1, wherein:

the predetermined property is protein conformation and/or immunogenicity.

58. (Withdrawn) The method of claim 57, wherein the LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

59. (Withdrawn, Currently Amended) The method of claim 1, wherein:

the predetermined property is protein amphipathic properties.

60. (Withdrawn) The method of claim 59, wherein the LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

61. (Withdrawn, Currently Amended) The method of claim 60, wherein, the *in silico* selection step comprises selecting a residue that is amenable to constituting a link or bridge between two regions of a protein is modified.

62. (Withdrawn) The method of claim 61, wherein the LEADs possess increased thermostability compared to unmodified target protein.

63. (Withdrawn) The method of claim 62, wherein the *in silico* selection step comprises selecting a residue in the protein that affects binding affinity to its cognate receptor.

64. (Withdrawn) The method of claim 63, wherein the LEADs possess either increased or decreased binding affinity to its cognate receptor compared to unmodified target protein.

65. (Withdrawn) The method of claim 1, wherein the modified target protein has a predetermined activity that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

66. (Withdrawn) The method of claim 1, wherein the modified target protein has a predetermined activity that is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein.

67. (Withdrawn) The method of claim 51, wherein the modified target protein ha a predetermined activity that is least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein.

68-78. Cancelled.

79. (Previously Presented) The method of claim 1, wherein the replacement amino acids are selected using Percent Accepted Mutations (PAM) matrices.

80. (Withdrawn) The method of claim 1, wherein the replacement amino acids are pseudo-wild type amino acids, whereby the resulting polypeptide retains activity of the unmodified polypeptide.

81. (Previously Presented) The method of claim 6, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

82. (Withdrawn) The method of claim 6, wherein the LEADs or super-LEADs possess increased digestibility compared to unmodified target protein.

83. (Withdrawn) The method of claim 6, wherein the LEADs or super-LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

84. (Withdrawn) The method of claim 6, wherein the LEADS or super-LEADs possess increased thermostability compared to unmodified target protein.

85. (Withdrawn) The method of claim 6, wherein:

each is-HIT target amino acid affects binding affinity to its cognate receptor; and

the super-LEADs possess either increased or decreased binding affinity to the LEADs or super-LEADs cognate receptor compared to unmodified target protein.

86. (Withdrawn) The method of claim 33, wherein the LEADs or super-LEADs possess either increased or decreased antigenicity compared to unmodified target protein.

87. (Withdrawn) The method of claim 33, wherein the LEADs or super-LEADs possess either increased or decreased amphipathic properties compared to unmodified target protein.

88. (Withdrawn) The method of claim 33, wherein the LEADs or super-LEADs possess increased thermostability compared to unmodified target protein.

89. (Withdrawn) The method of claim 33, wherein the LEADs or super-LEADs possess either increased or decreased binding affinity to the LEADs or super-LEADs cognate receptor compared to unmodified target protein.

90. (New) A highthroughput method for generating a protein or peptide molecule having a predetermined property or activity, comprising:

(a) identifying, within a target protein or peptide, one or more target amino acids amenable to providing the predetermined property or activity upon amino acid replacement, wherein:

the identifying of the one or more target amino acids in step a) is conducted *in silico*; and

each target amino acid locus is designated an *in silico*-HIT (is-HIT);

(b) identifying replacement amino acids to replace the residue at each is-HIT, wherein the replacement amino acids comprise all of the 19 remaining non-native amino acids;

(c) producing a collection of sets of nucleic acid molecules that encode candidate LEAD proteins, wherein:

each encoded candidate LEAD protein contains a single amino acid replacement;

each nucleic acid molecule in a set encodes the same candidate LEAD protein;

each candidate LEAD protein differs by one amino acid from the target protein or peptide, whereby the encoded candidate LEAD proteins in each set differ by one amino acid from the encoded candidate LEAD proteins in each of the other sets;

each set is separate from each and all other sets;

(d) individually introducing each set of nucleic acid molecules into host cells and expressing the encoded candidate LEAD proteins to produce sets of LEAD proteins, whereby:

each candidate LEAD protein in a set contains the same amino acid replacement;

the host cells comprise an addressable array such that each LEAD protein is expressed at a different locus in the array, and the identity of each candidate LEAD protein at each locus is known; and

(e) individually screening each set of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity of an unmodified target protein, wherein each such identified protein is designated a LEAD mutant protein.

91. (New) The method of claim 1, wherein the replacement amino acids in step (b) comprise all of the 19 remaining non-native amino acids.